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OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM *Eleusine indica* USING RESPONSE SURFACE METHODOLOGY

(Pengoptimuman Pengekstrakan Berbantu Gelombang Mikro bagi Sebatian Fenolik dari *Eleusine indica* Mengunakan Kaedah Gerak Balas Permukaan)

Angelica A. Angeles-Macalalad, Bryan John A. Magoling*, Jennielyn C. De Chavez, Love Angel H. Flores, Alona B. Intac

Department of Chemistry,
College of Arts and Sciences,
*Batangas State University-Pablo Borbon, Rizal Avenue, Batangas City, 4200 Philippine*s

**Corresponding author: bryanmagoling@gmail.com*

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Abstract

Eleusine indica belongs to the Pocaceae family and is abundantly found in many tropical countries. Because of its anti-malarial, antioxidant, anti-viral, and antidiabetic properties, among many others, studies are being aimed on developing time and cost-effective methods that could efficiently extract its active components. This study focused on the optimization of the microwave-assisted extraction (MAE) of phenolic contents from *E. indica*. A response surface methodology (RSM) using a Box-Behnken design (BBD) of experiment was employed to determine the optimized condition for the extraction method. The extraction was performed with three varying factors (ethanol concentration, microwave power, and irradiation time) and one response (total phenolic content or TPC). A maximum TPC of 74.81 ± 5.22 GAE mg/g was obtained through MAE, using 57.23% (v/v) ethanol with microwave irradiation of 217.77 W for 4.53 minutes. The optimized condition had an extraction yield of 11.62 ± 1.97%. The ethanolic *E. indica* extract obtained using the optimum condition contained mostly of triterpenes, saponins and glycosides; and moderately of flavonoids and tannins.

Keywords: *Eleusine indica*, microwave-assisted extraction, phenolic compound extraction, response surface methodology

Abstrak

Eleusine indica dari keluarga Pocaceae dan ia sering dijumpai di negara tropika. Oleh kerana sifatnya anti-malaria, antioksida, anti-viral dan antidiabetik, banyak kajian dijalankan bangi membangunkan kaedah yang berkesan dari aspek masa dan kos bagi tujuan pengkestrakan komponen aktif. Kajian ini memberi tumpuan kepada pengoptimuman pengekstrakan berbantu gelombang mikro (MAE) bagi kandungan fenolik dari *E. indica*. Kaedah gerak balas permukaan (RSM) mengunakan reka bentuk eksperimen Box-Behnken telah dibangunkan bagi penentuan keadaan optimum kaedah pengekstrakan. Pengekstrakan telah dijalankan Bersama tiga faktor (kepekatan etanol, kuasa gelombang mikro, dan masa penyinaran) dan satu respons (jumlah kandungan fenolik atau TPC). Nilai maksimum TPC ialah 74.81 ± 5.22 GAE mg/g diperolehi melalui MAE, mengunakan 57.23% (v/v) etanol bersama

217.77 W penyinaran gelombang mikro selama 4.53 minit. Keadaan optimum telah menghasilkan ekstrak 11.62 ± 1.97%. Ekstrak etanolik *E. indica* mengunakan keadaan optimum mengandungi triterpen, saponin dan glikosida, dan flavonoid dan tannin.

Kata kunci: *Eleusine indica*, pengekstrakan berbantu gelombang mikro, pengekstrakan sebatian fenolik, kaedaj gerak balas permukaan

Introduction

For many years, plants with medicinal properties have been widely used as sources of traditional medicines to treat various disease and illnesses. Since then, immense efforts have been put into the study and optimization of extracting active components from plants in the aim of increasing their medicinal potencies and applications. Plants are rich in many natural products, such as secondary metabolites, which possess valuable pharmacological activities. These secondary metabolites include phenols, phenolic glycosides, flavonoids, cyanogenic glycosides, saponins, unsaturated lactones, and glucosinolates [1]. Phenolic compounds have been found to have anti-inflammatory [2], anti-mutagenic [3] and antimicrobial activities [4]; exhibit chemoprotective [5]; and inhibit nitric oxide production [6]. Recent reports have found high phenolic compounds in *Eleusine indica* [4, 7, 8].

E. indica (also known as goose grass) is an invasive annual weed which is native in tropical and subtropical regions. It has been used as a traditional medicine for treating diarrhea and malaria [9]. It has also shown anthelmintic [10], antioxidant [4], anti-viral [11] and antidiabetic [12] properties. The crude chloroform and methanolic extracts of *E. indica* were reported to contain tannins, flavonoids, and alkaloids which exhibited anti-fungal and antibacterial activities against *Enterobacter aerogenes*, *Escherichia coli, Klebsiella agerogenes, Proteins vulgaris, Pseudomonas aerogenes, Staphylococcus aureus*, and unspecified *Streptococcus* and *Bacillus* species [13].

The phenolic compound extraction from *E. indica* is commonly performed using a conventional maceration method, which may involve different solvent systems (water, methanol, ethanol, ethyl acetate, or hexane) [14]. However, this extraction technique consumes high amounts of solvent, requires long extraction times, and impose a high risk of degrading the heat-labile constituents in the sample. To resolve these drawbacks, increasing studies are being done aiming to develop more efficient extraction techniques. Of these methods, the microwave-assisted extraction (MAE) shows one with the highest potentials. The application of MAE does not only reduce the amount of solvent and extraction time needed; it also improves the extraction yield with minimal degradation of bioactive compounds in the sample [15]. The amounts of phenolic compounds extracted from pineapple peelings using conventional (Soxhlet) and microwave-assisted methods have been compared and it was found that at a similar sample-to-solvent (ethanol) ratio, MAE required 6-fold less time to extract around 7-fold more phenolic content compared to Soxhlet method [16]. Further, the overall operational cost of microwave-assisted processes is estimated to be lower than those using conventional heating in terms of energy consumption and the overall process turnover time [17]. Hence, the use MAE could be a good alternative for extracting phenolic compounds in medicinal plants.

Few reports are available on the extraction of total phenolic compounds (TPC) from *E. indica*. These studies either used cold maceration [4], decoction [8], or ultrasonication [7] in their extraction procedures. The main factors affecting MAE efficiency include sample particle size, pre-leaching time, microwave power, extraction temperature, solvent to solid ratio, solvent composition, and extraction time/cycles [18].

For studying a process with multiple variables, an experimental design that can generate optimum conditions from a minimum number of experiments is highly desired. Response surface methodology (RSM) is a useful technique for studying the effects of important factors, unaccompanied or in combination with the involved processes [19]. It is also capable of generating a mathematical model of the possible interrelationship among the tested variables. Compared to other designs of experiments for RSM, such as Central Composite Design (CCD), the Box-Behnken Design (BBD)

requires fewer design points to study processes with a minimum of three levels and allows for studying processes with extreme ranges.

Achieving higher yields using safer and better extraction method (considering economic, environmental and practical dimensions) for the phenolic content extraction in *E. indica* using MAE presents many economic and medicinal potentials. Therefore, this study aimed to determine the optimum condition for the microwave-assisted extraction of phenolic compounds from *E. indica*, at varying ethanol concentrations, microwave power and microwave irradiation time using RSM with a BBD experimental design.

Materials and Methods

Chemicals and reagents

The Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), and acetic acid were purchased from Sigma-Aldrich (St. Louis, Mo). Gallic acid and ethanol were purchased from ATR Trading System (Metro Manila, PH). All other chemicals and reagents used in this study were analytical grade.

Sample collection

The *Eleusine indica* plants were collected from Barangay Dalig, Batangas City, Philippines. The samples were authenticated at the Bureau of Plant Industry, Department of Agriculture, Manila, Philippines with the certificate number of PLT-ID-CRPSD-0833-18.

Sample preparation

The collected plant samples were rinsed with tap water followed by distilled water to remove dirt from the surface. They were air-dried in a well-ventilated area at room temperature until a constant weight difference of ± 0.001 kg was obtained. The dried samples were crushed using an Astron BL153 food processor, and then sieved using a size 30 mesh screen. The sieved samples were transferred into air-tight glass containers and stored in room temperature for further analyses.

Microwave-assisted extraction of phenolic contents

Preliminary experiments were carried out to determine the working ranges of individual parameters, prior the RSM experiments. Ethanol was chosen as the solvent for the microwave-assisted extraction (MAE) process since it has been reported to extract higher concentrations of phenolic compounds compared to water, methanol, and acetone [16, 20, 21]. The determined parameter ranges for the MAE of the TPC from *E. indica* were investigated and optimized using a response surface methodology (RSM), following a BBD of experiment.

In here, the three selected variables (ethanol concentration, microwave power, and irradiation time) were varied at either a low, medium, or high level (coded as -1, 0, and +1, respectively). The measured TPC from each extraction experiment served as the responses. These responses were then used to generate a mathematical model for the process. The design matrix used in this study is summarized in Table 1.

Table 1. Independent variables and their coded levels for the Box-Behnken Design (BBD) of experiment

Factors	Code	Unit	Coded Variable Levels		
			-1	0	1
Ethanol concentration	A	% v/v	20	40	60
Microwave power	B	Watt (W)	210	350	490
Microwave irradiation time	C	minute (min)	4	7	10

The extraction process was performed based on the study of Pan *et al*. with some modifications on the pre-setting procedure [22]. Preliminary experiments were carried out to test and determine the working ranges of individual parameters (i.e., sample-to-solvent ratio, pre-leaching time, solvent concentration, microwave power, and irradiation time) for the phenolics extraction process. The three parameters with the highest individual effects on the phenolic extraction yield were then selected and their individual and combined interactions were studied further using RSM.

Prior to microwave treatment, the air-dried *E. indica* samples were subjected to a pre-leaching step by soaking them in varied concentrations (20, 40, or 60% v/v) of ethanol (20 mL/g dried sample) for 90 minutes. Using a MWX203 BL Whirlpool microwave oven (with eleven discreet MW power level settings ranging from 0 to 700 W), the mixtures were then exposed to varying microwave power levels (210, 350, or 490 W) for either 4, 7, or 10 minutes. After microwave treatment, the sample mixtures were cooled to room temperature, then filtered through a Whatman No. 1 filter paper (Whatman, Maidstone, Kent, UK). The filtrates were transferred into volumetric flasks and were diluted to a final volume of 100 mL.

***E. indica* extract sample preparation**

The TPC of the ethanolic *E. indica* extracts were determined using the Folin-Ciocalteu method [23]. In brief, 1 mL of each extract was transferred into a volumetric flask, followed by the addition of 5 mL of the Folin-Ciocalteu reagent and 4 mL of Na_2CO_3 solution, respectively. The solutions were mixed and then incubated (away from light) for 1 hour at room temperature, prior to absorbance read-out.

Gallic acid standard preparation

A 1 mL volume of each standard solution was transferred into a volumetric flask, followed by the addition of 5 mL of the Folin-Ciocalteu's phenol reagent and 4 mL of Na_2CO_3 solution, respectively. The solutions were mixed and then incubated (away from light) for 1 hour at room temperature, prior to absorbance read-out.

Spectroscopic analysis of standard and sample solutions

The absorbance of each of the prepared standard and sample solutions was measured at 760 nm using a UV-2700 UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The TPC in each of the original sample solutions were computed using the generated standard calibration curve equation and were expressed as the milligram of gallic acid equivalent (GAE) per gram of dried *E. indica* sample, as shown in the equation 1:

$$TPC = C1\ (V/w) \quad (1)$$

TPC is total phenolic content expressed as GAE (mg/g); C1 is the GAE concentration in the sample (mg/mL); V is the volume of extract in mL; and w is the weight of the initial dried plant sample used (g).

Extraction process optimization and validation

The optimization of the microwave-assisted extraction of phenolic compounds from *E. indica* was carried out using the Design- Expert® v10.0.8 (Stat-Ease Inc., Minneapolis, MN, USA) software. An analysis of variance (ANOVA) was applied to test the validity of the generated model. The adequacy of the fitted model was determined by evaluating the lack of fit and the coefficient of determination (R^2). The model's Fisher variation ratio (F-value), probability value (Prob > F), and adequate precision (AP) were also evaluated to test for its significance and adequacy.

Extraction yield of the optimized MAE

To obtain the dried *E. indica* extract, the ethanolic extract was concentrated using a rotary vacuum evaporator (Buchi rotavapor R-205) set at 280 rpm for 15 min. at 40°C. The extraction yield was calculated using equation 2 [24]:

$$\text{Extraction yield (\%)} = (\text{mass of dried extract / mass of } \textit{E. indica}) \times 100 \quad (2)$$

Phytochemical screening

Standard procedures for the phytochemical screening of the *E. indica* extract obtained from the optimized MAE condition were performed to identify and estimate the presence of its chemical constituents such as alkaloid, flavonoid, saponin, triterpenoid, steroid, tannins, and glycosides [25].

Test for Sterols (Liebermann-Burchard Test). About 1 g of the sample was dissolved in 2 mL of acetic acid. The soluble portion was collected and was kept on ice. One to two drops of H_2SO_4 was then added to the solution. The development of color from violet to blue or bluish-green indicates the presences of steroidal rings.

Test for Triterpenoids (Salkowski Test). A 0.5 g of sample was mixed in 2 mL of chloroform. Then, 3 mL of concentrated H_2SO_4 as dropwise added to the mixture. The development of a reddish-brown color indicates the presence of triterpenoids.

Test for Flavonoids (Shinoda Test). Magnesium ribbon fragments were mixed with 2 g of the sample. Then, a few drops of concentrated HCl were added. The development of a pink to red color indicates the presence of flavanoids.

Test for Saponins (Froth Test). Hot distilled water was used to dissolve 5 g of sample and the mixture was then filtered. The filtrate was vigorously shaken. The formation of a persistent froth that lasts at least 15 minutes indicates the presence of saponins.

Test for Glycosides (Fehling's Test). Hot distilled water (5 mL) was used to dissolve 1 g of sample and the mixture was then filtered. Into each of two test tubes, 2 mL of the filtrate was added. In the first test tube, 1 mL of HCl was added; nothing was added to the second test tube (control). Both of the tubes were placed in a water bath at 100ºC for 5 minutes, then cooled to room temperature. Anhydrous sodium carbonate was added to neutralize the sample until there was no more effervescence. To each test tube, 1 mL of freshly mixed Fehling's A and B (1:1) was added. The test tubes were then placed in a water bath at 100ºC for 2 mins. The formation of the brick red precipitate indicates the presence of glycosides.

Test for Tannins (Ferric Chloride Test). The dried extract was dissolved in hot water then filtered. Few drops of 5% ferric chloride solution were added. A dark green or bluish-black color indicates the presence of tannins.

Results and Discussion

Microwave-assisted extraction parameters and responses

A total of fifteen experiments were performed to generate a response surface model (RSM) for the extraction of phenolic contents in *E. indica* at varying ethanol concentration, microwave power, and microwave irradiation time. The experimental factors used in this study and their responses are listed in Table 2. Each value is the mean of triplicate analysis. The results from the experiment revealed TPC varying from 16.82±1.3 to 74.81±3.9 GAE mg/g.

Table 2. Experimental factors and their responses for the MAE of TPC from *E. indica*

Standard Number	Run Number	A: Ethanol Concentration (% v/v)	B: Microwave Power (W)	C: Irradiation Time (min)	TPC [a] (GAE mg/g)
1	2	20	210	7	25.67 ± 1.5
2	4	60	210	7	60.05 ± 3.6
3	12	20	490	7	35.20 ± 3.7
4	13	60	490	7	25.41 ± 1.8
5	11	20	350	4	34.53 ± 4.1
6	10	60	350	4	62.67 ± 1.4
7	3	20	350	10	25.55 ± 1.5
8	15	60	350	10	16.82 ± 1.3
9	7	40	210	4	74.81 ± 3.9
10	9	40	490	4	58.80 ± 4.5
11	8	40	210	10	30.93 ± 2.1
12	6	40	490	10	29.23 ± 1.8
13	5	40	350	7	44.46 ± 3.3
14	14	40	350	7	44.11 ± 2.1
15	1	40	350	7	41.80 ± 2.5

[a] TPC values are expressed as mean ± standard deviation (SD), calculated from three independent experiments.

Optimization of the MAE of phenolic compounds in *E. indica* using RSM

The responses obtained from the batch microwave-assisted extraction experiments, with respect to the variables used, were fitted with the second-order polynomial equation 3:

$$Y = b_o + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} b_{ij} X_i X_j + e_i \quad (3)$$

where Y is the predicted response, b_o is the constant coefficient, bi is the linear coefficient, b_{ij} is the interaction coefficient, b_{ii} is the quadratic coefficient, Xi and X_j are the coded values of the AC activation factors, and e_i is the error.

As shown in Table 3, an analysis of variance (ANOVA) was performed to determine which model-order best fit the responses for the process in study. The acceptability of the model was evaluated based on correlation coefficient (R^2) and standard deviation. The model fitting revealed that the microwave-assisted extraction of phenolic compounds in *E. indica* best followed a second-order response surface model.

Table 3. ANOVA of the quadratic model of MAE of TPC from *E. indica*

Source	Sum of Squares	df	Mean Square	*F*-value	*p*-value	Remark[a]
Model	3872.59	9	430.29	37.29	0.0005	sig.
A - Ethanol Conc.	242.11	1	242.11	20.98	0.0059	sig.
B - MW Power	229.26	1	229.26	19.87	0.0067	sig.
C - Irradiation Time	2056.96	1	2056.96	178.24	< 0.0001	sig.
AB	487.67	1	487.67	42.26	0.0013	sig.
AC	340.11	1	340.11	29.47	0.0029	sig.
BC	51.16	1	51.16	4.43	0.0891	not.sig.
A^2	385.08	1	385.08	33.37	0.0022	sig.
B^2	41.14	1	41.14	3.57	0.1176	not sig.
C^2	10.04	1	10.04	0.87	0.3937	not sig.
Residual	57.70	5	11.54			
Lack of Fit	53.50	3	17.83	8.49	0.1071	not sig.
Pure Error	4.20	2	2.10			
Standard deviation = 4.43				R^2 = 0.9600		
Coefficient of variance = 10.90				Adjusted R^2 = 0.9300		
Adequate precision = 17.077				Predicted R^2 = 0.8631		

[a] sig. = significant; not sig. = not significant

A high correlation coefficient value of 0.9600 was obtained in the present study, indicating that only 4.00% of the total variation is unexplained by the generated model.

The ANOVA results for the quadratic response surface model for TPC extraction gave an F-value of 37.29 and a corresponding probability > F that is less than 0.05, validating that the model is significant. For the model terms, probability factor (prob > F) values less than 0.05 are considered significant. In this work, model terms *A*, *B*, *C*, *AB*, *AC*, and A^2 were significant while *BC*, B^2 and C^2 were insignificant terms. To improve the model's efficiency, insignificant model terms were excluded from the study.

Adequate precision (AP) describes the ratio between signal and noise and is used to decide whether the

predicted model can be utilized to move along the design space [26]. The obtained AP ratio of 17.077 that is higher than the desired value of 4 suggested that the model presented in this work is efficient to predict TPC of the extract, within the established set of parameters.

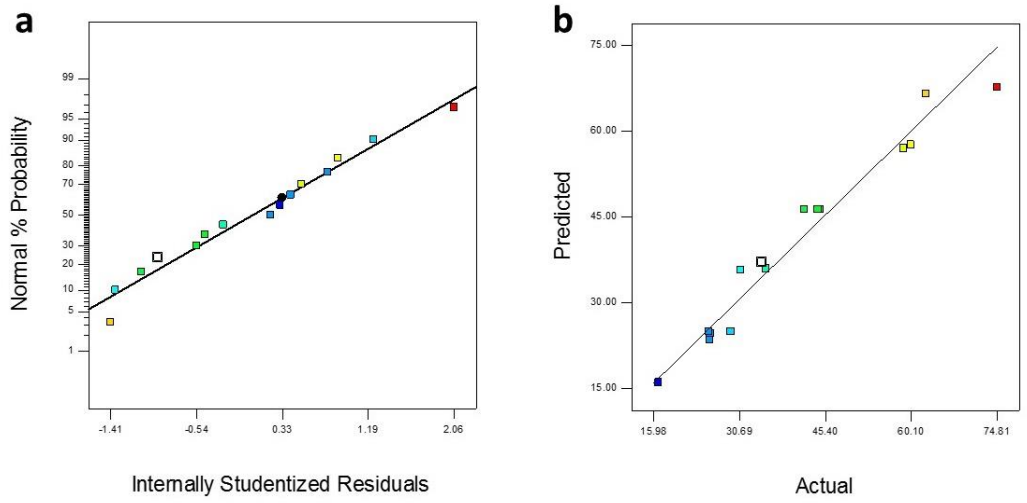

Figure 1. Plots of the (a) normal probability versus studentized residuals and the (b) predicted versus actual values for the MAE of TPC from *E. indica*

The normal probability plot of the studentized residuals for TPC extraction is illustrated in Fig. 1a. The linear plot illustrated adequate standard distribution, with minor scattering, between the residuals and the experimental points. Fig. 1b revealed good agreement between the experimentally-obtained and the model-generated responses. This indicated that the data generated by the model were valid and may represent that of the real system.

The final regression model, in terms of the coded factors for total phenolic content (TPC), is expressed in equation 4:

$$\text{TPC (GAE mg/g)} = 46.31 + 5.50A - 5.35B - 16.03C - 11.04AB - 9.22AC - 10.57A^2 \quad (4)$$

where TPC is total phenolic content expressed as GAE (mg/g), *A* is the ethanol concentration (% v/v), *B* is the MW irradiation power (210 to 490 W), and *C* is the MW irradiation time (4 to 10 min).

On the other hand, the final regression model, in terms of the actual factors for the total phenolic content (TPC), is expressed equation 5:

$$\text{TPC (GAE mg/g)} = -54.41274 + 4.84476(\text{ethanol conc.}) + 0.11950(\text{MW Power}) + 0.80238(\text{MW irradiation time}) - 3.94342\text{x}10^{-3}\,(\text{ethanol conc.})\,(\text{MW Power}) - 0.15368(\text{ethanol conc.})\,(\text{MW irradiation time}) - 0.02642(\text{ethanol conc.})^2 \quad (5)$$

Both equations 4 and 5 can be used for predicting the TPC yields along the design space. However, only the coded coefficients are proportional to the observed effect since the intercept in coded values is in the center of the design. In the actual values, the intercept usually lies far from the design space. The coefficients in terms

of actual factors account for differences in the factor ranges which may not be directly interpreted. Eq. 4 presents the relative effects of the model terms toward the TPC yield. Specifically, it could be observed that irradiation time exhibits the largest effect toward the TPC yield of the MAE process, while microwave power presented a comparable magnitude but contrasting effect as that of ethanol concentration.

The optimum extraction condition was established using Design-Expert® v10.0.8 (Stat-Ease Inc., Minneapolis, MN, USA) software. In the software optimization step, the goal for each operational condition (ethanol concentration, microwave power, and irradiation time) was set to be 'within' range while the response (TPC) was set as 'maximum' to achieve the highest possible yield. The highest predicted TPC was obtained at 52.23% v/v of ethanol, with microwave irradiation of 217.77 W for 4.53 min. with a predicted TPC of 75.22 GAE mg/g.

To further illustrate the relationships among parameters, ethanol concentration, microwave power input and irradiation time, toward the responses predicted by the model, three-dimensional (3D) surface response plots were generated, as shown in Figure 1.

In the plots shown in Figure 2, one variable was kept constant at the optimum value, while the two remaining parameters were varied within their experimental ranges. Figure 2a shows the 3D response surface plots of the combined effects of the ethanol concentration and microwave power, while the irradiation time was kept at the optimum value of 4.53 minutes. The predicted TPC in the *E. indica* extract showed an increasing trend with increasing ethanol concentration of up to 57.23%. Beyond this point, the TPC levels started to plateau which indicated that higher concentrations of the solvent had minimal effect on the TPC extraction. On the other hand, a decreasing trend in TPC yield was observed beyond the established optimum MW power around 218 W.

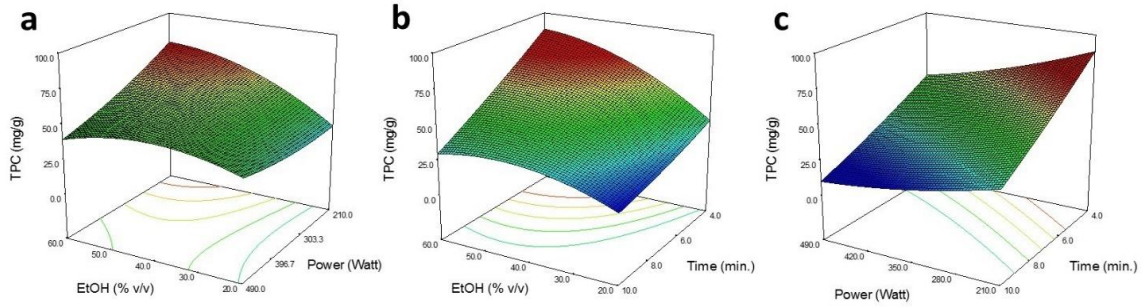

Figure 2. 3D response surface plots showing the predicted total phenolic content in the *E indica* extract as a function of (a) microwave power and ethanol concentration; (b) microwave irradiation time and ethanol concentration; and (c) irradiation time and microwave power.

Figure 2b shows the effect of the contact between ethanol concentration and microwave irradiation time on the TPC of *E. indica* when microwave power is fixed at 217.77 W. Increasing the ethanol concentration up to its optimum level showed positive correlation with the TPC in the extract, while a decreasing trend in TPC was observed as the irradiation time was increased above the optimum level of around 4.5 mins.

Figure 2c demonstrates the contact between microwave power and irradiation time on the TPC value at a fixed ethanol concentration of 57.23%. The 3D plot showed that the TPC level was at its highest when both parameters were set near their lowest levels.

Experimental validation of the predicted TPC from the optimized MAE condition

The predicted TPC in the *E. indica* extract obtained using the optimized MAE condition was validated by comparing experimentally obtained TPC values with the predicted TPC value. Table 4 shows the comparison of the experimental and the predicted TPC in the *E. indica* extracts obtained from the optimized MAE condition.

Table 4. Experimental validation of the predicted TPC obtained from optimized MAE condition

Optimized MAE Condition			Experimental TPC (GAE mg/g)	Mean Exp. TPC ± s.d. (GAE mg/g)	Predicted TPC (GAE mg/g)	% Error
Ethanol Conc. (% v/v)	MW Power (W)	Irradiation Time (min.)				
57.23	217.77	4.53	71.00 72.72 80.75	74.81 ± 5.23	75.22	0.541

As shown in Table 4, the obtained experimental TPC value of 74.81 ± 5.23 GAE mg/g was in close agreement with the predicted TPC of 75.22 GAE mg/g. A computed relative percent error of 0.541% between the predicted and obtained experimental value was observed. This validated the reliability of the generated predictive model and the goodness-of-fit with the experimental results.

Extraction yield of the *E. indica* extract obtained using the optimized MAE condition

Table 5 shows the extraction yield of the ethanolic *E. indica* extracts obtained using the determined optimum MAE condition. The mean extraction yield was determined to be 11.62 ± 1.96%.

Table 5. Extraction yield of the ethanolic *E. indica* extract obtained using the optimized MAE condition

Sample	Extraction Yield (%)
Trial 1	10.85
Trial 2	10.16
Trial 3	13.86
Mean ± s.d.	11.62 ± 1.96

Qualitative determination of phytochemicals in the *E. indica* extract

Based on Table 6, the ethanolic *E. indica* extract obtained using the optimized microwave-assisted extraction condition showed was abundant in triterpenes, saponins and glycosides. On the other hand, moderate amounts of flavonoids and tannins were observed. Sterols were not detected in the extract.

Table 6. Phytochemicals present in the *E. indica* extract obtained using the optimized MAE condition

Secondary Metabolite	Presence [a]
Sterols	(-)
Triterpenes	(+++)
Flavonoids	(++)
Saponins	(+++)
Glycosides	(+++)
Tannins	(++)

[a] presence is reported as either (-) absent, (+) traces, (++) moderate, or (+++) abundant

In this paper, the microwave-assisted procedure for the extraction of phenolic contents in *E. indica* was optimized, aiming to maximize TPC extraction yield while minimizing both the processing time and solvent consumption. The established optimized condition yields high amounts of extracted TPC, at a quicker processing time compared to conventional maceration methods.

In the predictive model equation, irradiation time showed to have the largest effect toward the TPC yield of the MAE process, while microwave power presented a contrasting effect as that of ethanol concentration. In the MAE of TPC from pomegranates, it was observed that more phenolics could be extracted using 50% ethanol than 70% [27]. It was associated with the fact that both the dielectric constant and dielectric loss factor of water are higher than those of ethanol. Under the same microwave power, the solvent with a fair ratio of ethanol and water may absorb more thermal energy enough to increase diffusion of phenolics. Based on the 3D response models (Figures 2a-c), the amount of TPC extracted from the sample start to decrease when the sample was exposed to higher microwave power for longer periods of time.

Introduction to microwave energy could strengthen the molecular interaction between the electromagnetic field and sample and improve the efficiency of the extraction process [28]. However, the observed decrease in TPC yield when the sample is exposed to MW levels that are beyond the established optimum could be caused by degradation of the desired phenolic compounds at higher levels of microwave power. Similar observations were seen in the findings of Peng et al. and Zheng et al. [29, 30].

While the nature of solvents used for MAE greatly contributes toward extraction efficiency, polar solvents such ethanol and water can get rapidly heated as they become exposed to too high levels of microwaves, resulting to the degradation of thermo labile phenolics and/or coextraction of other unwanted compounds in the sample [31]. More so, exposure to too much MW energy input for an excessive period could cause the sample mixture's temperature to extremely and abruptly elevate, initiating the rapid break down of the plant matrix. Hence, the MW power input and irradiation time must be maintained at the optimum level to achieve a selective MAE process and avoid solute degradation due to abrupt cell bursting from pressure buildup [32].

The optimum MW power level and irradiation time established for the MAE of TPC in *E. indica* both lie near the lower ends of the studied ranges. While this could indicate a possibility that the method may still be further optimized by exploring lower levels of MW power and shorter irradiation times, it was not done in this present study because of a limitation in the MW irradiation mechanism of the commercial microwave oven used.

The ethanolic *E. indica* extract obtained in this work contained different phenolic compounds such as triterpenes, saponins, glycosides, flavonoids, and tannins which are comparable to the finding in the study of *E. indica* by Morah and Otuk [13]. Phenolic compounds have been identified to exhibit many medicinal properties. The current optimized procedure was able to extract high amounts of secondary metabolites from *E. indica*, which can be further processed and used for medicinal applications.

Conclusion

The optimization of microwave-assisted extraction (MAE) of phenolic contents from *E. indica* was investigated in this present work. A second-order regression model for predicting the total phenolic contents (TPC) at varying ethanol concentration, MW irradiation power, and MW irradiation time was developed, with a high R^2 value of 0.9600. The diagnostic plots for the generated model demonstrated good agreement between the predicted and experimentally–obtained TPC extraction, proving the validity of the predictive model. Analysis of the 3D response plots derived from the model revealed interactions among the three parameters. Optimization was carried out using RSM following BBD with three variables. The optimized conditions for the MAE of TPC was successfully established through initial contact of dried *E. indica* samples to 57.23% v/v ethanol, followed by MW irradiation at 217.77 W for 4.53 min.

This resulted to the TPC yield of 74.81 ± 5.23 GAE mg/g. By optimizing the ethanol concentration, microwave power, and irradiation time for MAE, we were able to extract high amounts of phenolic compounds from *E. indica* in a short processing time. The obtained extract in this work contained abundant amounts of important secondary metabolites that could be further processed and used for medicinal applications.

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